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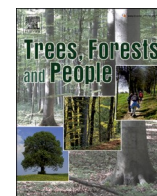
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Morphological, nutritional and medicinal traits of wild mango (*Mangifera Sylvatica* Roxb.): Implications for increased use and options for cultivar development

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ABSTRACT

Mangifera sylvatica Roxb. is an underutilised and threatened wild fruit species found in Bangladesh, which is highly valued by local people as a source of fruit and is an important source of nutrition. As part of a feasibility study of the domestication and cultivar development potential of *M. sylvatica*, a preliminary study examined the morphological traits (fruit, kernel and pulp mass), nutritional profile (carbohydrate, sugar, pH, fat, protein, mineral and vitamins) and medicinal traits (total phenolic and phenolic profiling). The fruit of *M. sylvatica* is small ($27.00\text{g} \pm 7.03\text{g}$) with a comparatively bigger kernel fruit (40% of its body weight). *M. sylvatica* fruit pulp has been proved to be a good source of carbohydrate, Vitamin C, sodium (Na) and potassium (K) and also has good medicinal properties (mangiferin and quercetin). The kernel is also a rich source of carbohydrate and has a good fatty acid profile (rich in stearic and oleic acids) consistent with cocoa butter, which indicates its potential to be used in the chocolate and confectionery industry. There is continuous variation in these traits, indicating opportunities for multiple trait cultivar development targeted at the food and pharmaceutical industries. The information generated in the study can be used as a stimulus to the process of domestication and to encourage widespread use of the species, which will ultimately help to conserve this wild underutilised fruit species.

1. Introduction

Forests contribute to the food security and livelihoods of more than 1.6 billion people worldwide (FAO, 2016) by providing different timber and non-timber products (Tchoundjeu et al., 2010; Leakey et al., 2005b). Around 30% of the world's forests are used primarily for production of wood and non-wood forest products (Vinceti et al., 2013). Recently, forest tree improvement for non-timber forest products (fruits, nuts, resin, etc.) has been implemented from high-value multi-purpose trees (Tchoundjeu et al., 2010). However, trees that produce edible fruits are of special interest, because fruits trees can build a safety buffer and often can serve as important sources of vitamins, minerals and phytochemicals that improve human health conditions (Leakey, 1999). Intake of vegetables and fruits can thus lead to reduction of cardiovascular diseases (Hu, 2003; Ikram et al., 2009), certain cancers (Ikram et al., 2009;

Riboli and Norat, 2003), immune system problems, arthritis, inflammation and brain dysfunction (Leong and Shui, 2002). The nutritional profiles of fruits from wild underutilised fruits in terms of micro-nutrients, fat, fibre and protein often make them an important supplement to staple foods (Leakey, 1999). Throughout the tropics there are many tree species presently used for the harvesting of fruits and other non-timber forest products at the local scale, but which are still not domesticated to the extent that they can provide products that can be traded beyond the local scale (Leakey and Tchoundjeu, 2001). For domestication of such multipurpose species, it is important to identify elite individuals in the wild or semi-domesticated population (Leakey and Page, 2006). Characterising phenotypic variation is a pre-requisite for cultivar development, which would benefit farmers through increasing productivity and product uniformity (Leakey et al., 2005b).

In Bangladesh, there are also several underutilised tree species such

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as water caltrop, palmyra palm and wax jambu (Rahim et al., 2009), used as fruit, that have not undergone systematic improvement though development of varieties. Their nutritional value and potential for improvement though selections are typically poorly investigated, and many of these species may, therefore, represent underutilised species suitable to combat malnutrition. Little attention has been given to increase the use of underutilised fruits to reduce malnutrition in Bangladesh (Rahim et al., 2009). Additionally, important contribution of fruit trees to many farmers' livelihoods and nutrition is often not acknowledged in national reporting (Shajib et al., 2013). Many such indigenous fruit species are declining and there is an urgent need to conserve them (Shajib et al., 2013; Rahim et al., 2009). However, prioritising conservation efforts requires information on the positive attributes of such species, which is often lacking. Many of these species could hold the potential of being integrated into agroforestry systems since they are already used by local communities, but development of improved varieties through domestication may be required in order to make planting attractive. The first step in a domestication programme is the sourcing of germplasm through identification of elite individuals from the wild population (Leakey and Page, 2006). However, very few studies have been conducted on the selection and breeding of superior trees. Since selection of the mother tree is the first step towards cultivar development, studies of tree-to-tree variation in fruit traits, including phenotypic and nutritional characters, is important (Leakey et al., 2005b; Leakey and Page, 2006) and is, therefore, the topic of the present study.

In Bangladesh, there are 47 edible wild forest fruits available (Das, 1987), an important one being *M. sylvatica* Roxb. (wild mango), which is genetically very close to *Mangifera indica* (common mango). A close relationship between the two species has been reported by Mukherjee (1950, 1957) and Nishiyama et al. (2006), which indicates that *M. sylvatica* may have the potential to fulfil nutritional and livelihood needs. This species is declining at an alarming rate due to a variety of factors, including legal or illegal logging, shifting cultivation and forest fires associated with clearance for shifting cultivation, which have a negative impact on regeneration (Baul et al., 2016; Dewan, 2009). In Bangladesh, this species is one of 16 recommended species mainly used in the plywood industry (Sattar, 2006). So, overall, there is tremendous pressure on *M. sylvatica* and no conservation effort except for some minor initiatives by the Bangladesh Forest Research Institute (BFRI, 2013). Over time, the habitat of this species has shrunk due to an ever-increasing demand on land for cultivation, industry, human habitation, overexploitation, illegal logging and encroachment (Baul et al., 2016). Furthermore, natural hybridisation may take place between *M. indica* and *M. sylvatica* in Southeast Asia (Mukherjee, 1950; 1957), which may potentially erode the genetic base of *M. sylvatica* if *M. indica* varieties are planted abundantly in the landscape. Based on the above background, the objective of the present study is to quantify the phenotypic and nutritional value of the species with focus on phenotypic tree-to-tree variation and implications for identification of superior clones to be propagated and tested for cultivar development. The study was conducted in the hilly region of Bangladesh and more specifically addressed the following research questions.

- 1 How large are the variations in fruit size, pulp and kernel weights between trees?
- 2 What are the nutritional properties of *M. sylvatica* fruit and to what extent does this vary between individuals?
- 3 Is there variation in phenotypic and nutritional properties among different sites?

2. Materials and methods

2.1. Study area

M. sylvatica is known as Jangli-am, Kosh-Am, Garey-Am, Lakkhi-Am,

Bon Am, Baitta Am, Chuchi Am and Guti Am in Bangladesh, and its most common name is Uriam. The species occurs mainly in the tropical humid forests, subtropical rainforests/woodlands and tropical dry forests/woodland of the Indo-Malayan bio-geographic region (Udvardy, 1975). It is native to India, Bangladesh, Thailand, China, Cambodia and Myanmar (Fig. 1). In Bangladesh, *M. sylvatica* is distributed in the hilly areas such as Chittagong, the Chittagong Hill Tracts (CHTs), Cox's Bazar and Sylhet (Fig. 1). *M. sylvatica* prefers 'Brown Hill Soils' (brown hill soils are drained soils with a yellow-brown to strong-brown Dystric Cambisols) that are found in the hilly regions and vary from brown sandy loam to clay loam (FRA, 2000). For the present study, samples were collected from Chittagong, Cox's Bazar and Sylhet. The CHTs have the highest elevation compared to other sites. The species generally grows in areas where average rainfall ranges from 2600–3000 mm/year. In the Sylhet division, the rainfall range is high. The optimum growing temperature for *M. sylvatica* is 22°C–30°C and the temperature of Cox's Bazar is comparatively higher than Sylhet, Chittagong and the CHTs.

2.2. Sample collection, preparation and analysis

Samples of fresh and ripe fruits were collected from three geographic locations (Cox's Bazar, Chittagong and Sylhet) in Bangladesh from April to May 2013. A total of fifteen trees from three sites were selected (five from each site). Ten fruits per tree were collected randomly throughout the canopy. The following fruit traits were assessed: fruit length, fruit width, fruit weight, peel weight, kernel weight and pulp weight, which were measured directly in the field using a portable electric balance and metre tape. The number of fruits per kg was recorded for fifteen trees (five trees per site) and the yield was recorded for three trees from the Cox's Bazar site. However, the approximate fruit yield was recorded for fifteen trees at three sites (five trees per site). For the nutritional analyses, fruits were collected from the same trees and refrigerated (at -4 °C) soon after collection. All fruits were then transferred to Bangor University in the UK for further analysis. Fruits were subsequently cleaned and separated into peel, pulp and kernel and stored in a freezer at -20°C. The frozen pulp samples were freeze dried and stored in air-tight bags and kept in the freezer at -20°C before further analysis. These samples were used for proximate analysis (dry matter content, moisture content, ash content, crude fat, crude fibre, crude protein, carbohydrate and energy). Apart from that, total soluble sugar (using refractometer), pH (using pH meter), Vitamin A (using HPLC-UV), Vitamin C (using HPLC-UV), macro and micro minerals (using total X-Ray fluorescence analyser), total phenolics (using folin-ciocalteu reagent) and phenolic compound were identified (using HPLC-UV detector), and fatty acid methyl ester analysis (using gas chromatography) was also analysed. Details of the methods used are given below.

2.2.1. Method for proximate, vitamin, mineral analysis

Determination of Moisture and ash content. Samples of 0.2g mango pulp and 1g of kernel was oven dried at 105 °C for three hours and dry matter and moisture content were determined. Samples were subsequently put in a muffle furnace at 600 °C for six hours and total ash content was recorded (Jaafar et al. 2009).

Determination of Crude fat. About 0.5g of mango pulp sample was weighed and 150ml of petroleum ether added to the flask and refluxed for four hours. The flasks were removed and dried with a rotary evaporator (Buchi Rotavapor R-114). Weight was recorded as crude fat content. For kernels, 4g of frozen mango kernel sample was weighed and then finely chopped, a solution of 2:1 = Chloroform:Methanol, ten times the weight of the mango kernel was added to the chopped mango kernels in a conical flask and homogenised using Ultra-Turrax (14A Ultra Turrax T25). The homogenised mixture was filtered three times and evaporated using a rotary evaporator (Buchi Rotavapor R-114) at 40 °C. Finally, the

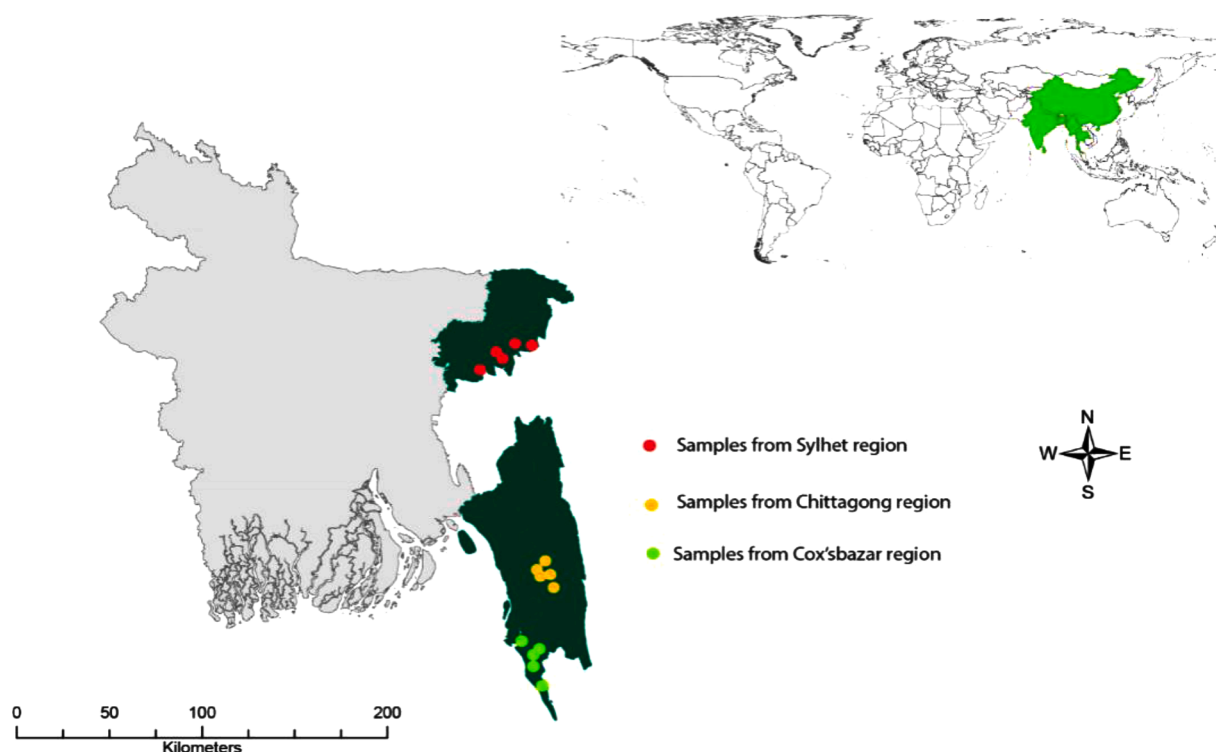


Fig. 1. Study area map with sampling location and world distribution of *M. sylvatica*.

weight was recorded as crude fat content.

Determination of Crude fibre. About 0.5g of mango pulp and kernel sample was sealed in fibre filter bags (ANKOM filter bags) which were put into 250ml conical flasks. Later, 200ml of 1.25% sulphuric acid solution was added into the conical flask and heated for 30 min and washed until traces of acid were detected using pH paper. After that, the acid extracted in the filter bags were transferred into 250ml conical flasks and again 200ml of 1.25% NaOH solution was added. The sample was heated for 30 min and washed with water until trace of base was detected by pH paper. The filter bags were oven dried for two hours at 105 °C (Sanyo convection oven MOV-212F). After that, the samples were transferred into pre-weighed crucibles and placed into a muffle furnace (Carbolite CWF 1200) at 600 °C for six hours and the weight of crucible was recorded (Nwanekezi et al. 2010).

Determination of Crude protein. Mango pulp and kernel powdered samples were oven dried (Sanyo convection oven MOV-212F) for two hours at 105 °C and a 0.1g sample was taken for nitrogen analysis. Samples were wrapped in tin foil and analysed using a Leco CHN 2000 analyser. The results were converted to protein content using a 6.25 conversion factor (FAO, 1992).

Determination of Carbohydrate and energy value (calories). Carbohydrate was determined by the following formula (Nwanekezi et al., 2010). Carbohydrate content (%) = 100 – (ash + dietary fiber + fat content + protein contents) %. Energy value was calculated using the formula Energy value (KJ/100 g) = [(% available carbohydrates x 17) + (% protein x 17) + (% fat x 37)].

Determination of total soluble solids, pH. Fresh mango pulp was used for this analysis. Total soluble solids (TSS) were determined using a hand refractometer in terms of °Brix. pH was measured using a pH meter. A total of 150 fruits were used from 15 trees (10 fruits from each tree and five trees from each provenance (sample area) to measure TSS and pH.

Vitamin analysis of *M. sylvatica* fruit pulp. Mango pulp samples were freeze dried and then powdered using ball mill. A 0.5g sample was first extracted with 16ml of 10mM ammonium acetate/methanol 50:50 (v/v) containing 0.1% BHT. After 15 min of shaking to achieve good sample dispersion in the extraction liquid, samples were placed in an ultrasound bath for 15 min. Bath temperature was controlled to 25 °C. The samples were centrifuged at 14,000 rpm for 15 min and the supernatant was collected. Three millilitres of the supernatant was concentrated into a N₂ stream and 1ml of 10mM ammonium acetate was added to the dried sample. This final sample was injected into a HPLC–UV system to determine the Vitamin C content. The solid residue from the first extraction was re-extracted with 12ml ethyl acetate containing 0.1% BHT, kept in the ultrasonic bath for 15 min. The samples were centrifuged (14,000 rpm for 15 min) and 3ml supernatant dried under a N₂ stream. Finally, the residue dissolved in 1ml of ethyl acetate and injected in a HPLC–UV system to monitor the Vitamin A content (Santos et al. 2012).

Mineral analysis of *M. sylvatica* fruit. For Sodium (Na) analysis, 0.2g of dried mango pulp and kernel sample was ashed in a muffle furnace (Carbolite CWF 1200) at 600 °C for six hours. After that, cooled ash samples were dissolved in 1ml of 20% HCl solution and 9ml of deionised water was added. Where necessary, the solution was filtered through acid-washed filter paper and finally analysis was done by flame photometer (Sherwood 410). For Potassium (K), Calcium (Ca), Phosphorus (P), Sulphur (S), Chlorine (Cl), Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu) and Nickel (Ni), total X-Ray fluorescence analysis was done. Here, oven dried samples were ground as fine powder using a ball mill and stored in airtight bags. A 0.02g sample was transferred to Eppendorf tubes and 1ml of Triton solution and 10 µl of Ga (1000 mg/l Ga internal standard) were added to the suspension. Samples were mixed well using a vortex and immediately after vortexing 10 µl of sample was pipetted into the center of a siliconised disc before the powder settled out of suspension. Samples were dried on a hot plate and assembled in the sample cassette. The first position of the cassette was reserved for Ga standard disc. All the samples were then run in the total

X-Ray fluorescence machine.

2.2.2. Method for medicinal properties analysis (total phenolics and phenolic profiling)

Sample collection and preparation. Only mature ripen fruits samples were considered for total phenolics and phenolic profile analysis. Mango pulp sample was freeze dried using freeze dryer and samples were kept in air tight poly bags for further analysis. For mango kernel samples were oven dried with air flow at 60 °C for 72 hours and then pulverized by blending to a homogeneous powder prior to extraction. All the dried mango kernel samples were stored in vacuum-sealed plastic bags at -20 °C until use. Finally, different fruit fractions were subjected to sequential extraction procedure.

Chemicals used. All the chemicals were purchased from Sigma Aldrich (Germany) and Extrasynthese (France). Folin-Ciocalteu reagent used for measuring total phenolics and gallic acid was used as standard purchased from Sigma Aldrich. The standards used for the identification of phenolic compounds were as follows: quercetin, keamferol, mangiferin, cinnamic acid, p-cumaric acid, syringic acid, tannic acid, chlorogegic acid, proto-catechuic acid, rhamnatin, gallic acid, benzoic acid, ellagic acid, ferulic acid, caffeic acid, and vanillic acid. All aqueous solutions were prepared in distilled water and solvents used for analysis were of HPLC grade.

Determination of total phenolic content in mango pulp, peel and kernel. The total phenolic content of the extract was determined colorimetrically, using the Folin-Ciocalteu method, as described by Singleton, Orthofer, and Lamuela-Raventó's (1999). For determination of total phenolics for mango pulp (0.5 g) and kernel (0.05g) samples were extracted using 20 ml of methanol: water (60:40 v/v) as described Ribeiro et al 2008. The mixture was centrifuged at 10000 rpm for 10 min. An aliquot of 0.1 ml of the extract were added to 0.1 ml of Folin-Ciocalteu reagent, followed by addition of 0.1 ml of an aqueous 7.5% solution of sodium carbonate. The mixture was stirred in a vortex machine and allowed to stand for 30 min. The absorbance measured at 765 nm using spectrophotometer. A blank sample consisting of water and reagents was used as a reference. The results were expressed as milligrams of gallic acid equivalents (GAE) per kg of dry matter. All the measurements were evaluated in triplicate.

Identification of phenolic compounds. For identification of phenolic compounds Solid Phase Extraction (SPE) was done from fresh mango pulp. Phenolic compounds were identified using HPLC-UV detector. For Solid Phase Extraction 20 ml supernatant injected through the C8 Column. Methanol (2ml), 5% Formic acid in water (2ml) used for elution of sugar. Later, 5% Formic acid in Methanol used for elutions of poly-phenols and collected in eppendorf tube for HPLC for profiling (Akhter 2012). This HPLC consist of C18 column and UV detector. The phenolic compounds done at 280nm. The mobile phase consisted of 5% formic acid in Water (Solvent A) and 95% MeOH as solvent B. The gradient program will be as follows: 5-90% B with 60 min and at 10 min will be the end of the flow (5% B and 0.5 ml/min flow rate). 10 µl samples will be injected into HPLC at a flow rate of 0.5 ml/min for phenolic fingerprints.

2.2.3. Method for fatty acid analysis

The fatty acid composition of kernels was analysed by g GC-MS. Kernels were separated from flesh and peel and the fresh kernel was used for fatty acid analysis. Frozen, chopped kernel was added to a mixture of 2:1 = Chloroform: Methanol and homogenised using an Ultra-Turrax. The homogenous mixture was filtered three times and evaporated using a rotary evaporator (Buchi Rotavapor R-114) at 40 °C. The butter produced was dissolved in methanol and chloroform (1:1) using a rotary evaporator and transferred into fresh vials. Afterward, 0.1

ml of solution mixture was placed in a 5ml vial for drying while stirring. Then, 1ml of heptane and 0.05ml of 1N Methanolic NaOH was added and stirred at 50 °C for two minutes. After two minutes, when the two layers were separated, the supernatant was collected and transferred to GC- vials for GC-MS analysis.

2.3. Statistical analysis

Descriptive statistics were derived using Minitab 17. For nutritional analysis, means, standard error (SE) and one-way ANOVA were performed. For phenotypic variation, the extent of trait variation between sites was determined from average and standard error for each trait (fruit length, fruit width, fruit mass, peel mass, kernel mass and flesh mass). Correlations between measured fruit traits were calculated based on tree average. Nested ANOVA was conducted to find the percentage of variation of different fruit traits at threelevels (between site, within site and within trees). Finally, a linear regression model ($Y = a + bX$) was developed between fruit weight, pulp weight and kernel weight.

3. Results

3.1. Morphological traits

M. sylvatica is a small mango fruit with a big kernel of more than 40% its body weight (Fig. 2). The mean fruit weight varied among trees from 17.85g to 35.96g (Table 1), while the kernel weight and the pulp weight varied from 7.29g to 15.02g and 4.96g to 11.63g (Table 1), consequently. It was also observed that the biggest fruits were found from the Cox's Bazar site and the Sylhet site produced second largest fruits. 67% of fruit weights were above 25g, kernel weights were above 10g and pulp weights were above 5g, and 77% of fruit lengths and widths were above 3cm and 2cm (Supplementary Fig. 1). The variation within sites was significantly higher ($P=0.01$) than between sites, indicating that a sample of ten fruits per tree and five trees per local site was sufficient to characterise the morphological variation (Table 2). Tree DBH and other fruit traits were weakly correlated, whereas fruit weight, pulp weight and kernel weight were strongly correlated with fruit length and fruit width, suggesting that the indirectly measurable traits can be measured through the directly measureable traits (Table 3). There were highly significant and strong relationships between fruit weight, pulp weight and kernel weight. Yield of pulp weight and kernel weight (g) could be predicted from fruit weight (g) by utilising the following model.

$$\text{PulpWeight} = 0.3919(\text{Fruit Weight}) - 2.1559, (R^2 = 0.973)$$

$$\text{Kernel Weight} = 0.3714(\text{FruitWeight}) + 1.0415, (R^2 = 0.973)$$

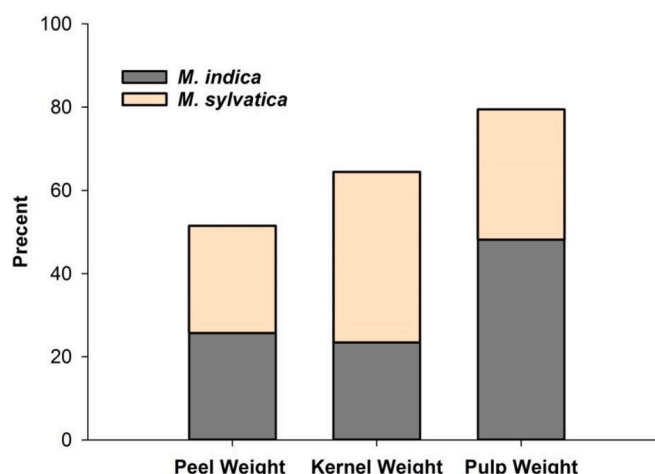


Fig. 2. *M. sylvatica* and *M. indica* fruit proportion weight.

Table 1
Morphological characteristics of fruits and components of *M. sylvatica*.

Variables	Local provenance Site1:(Cox's Bazar) Mean±SE	Site 2:(Chittagong) Mean±SE	Site3:(Sylhet) Mean±SE	All average Mean±SE
Fruit length (cm)	4.88±0.21	3.85±0.40	4.79±0.58	4.51±1.16
Fruit width (cm)	3.28±0.19	2.79±0.37	3.05±0.38	3.04±0.78
Fruit weight (g)	35.96±3.43	17.85±3.08	27.84±4.51	27.22±7.03
Peel weight (g)	8.87±0.98	5.23±0.92	6.97±1.19	7.02±1.81
Kernel weight (g)	15.02±1.14	7.29±1.16	11.14±1.39	11.15±2.88
Pulp weight (g)	11.63±1.59	4.96±1.16	8.95±1.98	8.51±2.20
Fruit: Pulp	3.17±0.17	4.07±0.69	3.95±0.96	3.73±0.96
Fruit: Kernel	2.38±0.08	2.45±0.15	2.42±0.18	2.42±0.62
Pulp: Kernel	0.77±0.07	0.67±0.10	0.74±0.13	0.73±0.19

N.B. SE (Standard Error)

3.2. Nutritional and medicinal properties

M. sylvatica fruits were, on average, juicy (85±0.57% moisture), low in carbohydrate (1.95%), rich in fibre (2%), Na (54.58 ± 9.97 mg/100g), K (172.76 ± 13.98 mg/100g), Vitamin C (41.51 ± 6.06 mg/100g) (Table 4, Table 5). The peel of this wild mango is thin (7.02 ± 1.81g) and contains high amounts of minerals (Na, K, Fe, Zn and Mn), which can be utilised as well (Table 5). *M. sylvatica* fruit (kernel) is a rich source of carbohydrate (20.36%), fibre (9.93%), fat (11.57%), minerals (K and Fe) and also very rich in medicinal compounds. Wild mango kernel contains higher amounts of phenolic compounds (115.44 ± 1.53mg/100g) compared to pulp (23.77 ± 0.30mg/100g) indicating that the kernel can be utilised for medicine purposes (Supplementary Fig. 2). Wild mango pulp is a source of mangiferin, quercetin and kaempferol (Fig. 4, Table 6).

4. Discussion

Morphological traits have been used traditionally to obtain information on variation within species. Continuous variation in fruit traits has important implications for domestication, suggesting opportunities for cultivar development through identification of elite individuals (Leakey and Page, 2006). Moreover, the domestication of wild fruit species depends on the expansion of the market demand for non-timber forest products (Leakey, 1999). So, it is recommended that selection of elite trees should not be based only on morphological traits, but also nutritive traits should be given priority. Many recent studies have documented variation in fruit traits in fruit tree species (Abasse et al., 2011; Assogbadjo et al., 2011; Fandohan et al., 2011; Gouwakinnou

et al., 2011). In Bangladesh, very little information is available on morphological and nutritional traits for many wild fruits species. This pioneer study quantifies variation in fruit traits of wild mango (*M. sylvatica*) and provides basic knowledge on the range of variation of several morphological and nutritional fruit traits within and between local sites of Bangladesh. The findings of this study highlight the opportunity to exploit natural populations of *M. sylvatica* for selection and identification of superior quality trees for production (pulp or kernel) and the development of suitable cultivars for domestication programmes (Leakey et al., 2004; Bationo et al., 2008; Allendorf and Luikart, 2007). Continuous variation was observed for the morphological fruit traits of *M. sylvatica*, which is important for the selection of elite trees. Selection of trees with an average fruit weight of 27g would result in considerable improvement in the quality and uniformity of marketable products. Screening a larger number of trees would almost certainly allow even greater benefits to be achieved, with the possibility of raising average fruit weights to 30g. However, to identify elite trees, it is recommended to include at least two morphological traits. Therefore, it will be worth including average fruit length (4.50 cm), which is strongly correlated with pulp weight and kernel weight (Table 3). Although between-site variation was found to be relatively high, particularly for fruit weight, pulp weight and kernel weight, within-site variation was prominent. Similar findings have been reported for African plum (*Dacryodes edulis* Lam.) and *Sclerocarya birrea* (Gouwakinnou et al. 2011; Leakey et al. 2004). Limiting the selection process based on morphological traits could lead to the loss of some essential market prospects. Therefore, it is better to consider nutritional traits for the selection of elite trees. From the proximate analysis, we found that the tree-to-tree variations were not too large in moisture and fibre for fruit pulp. Moisture content is an

Table 2
Variation of traits (in percentage) from nested analysis of variance ($P=0.05$).

Source of variation	Fruit length	Fruit width	Fruit weight	Peel weight	Kernel weight	Pulp weight
Among provenance	10.92 ($P=0.20$)	0.00 ($P=0.56$)	45.73 ($P=0.01$)	24.92 ($P=0.08$)	57.18 ($P=0.00$)	33.77 ($P=0.03$)
Within provenance						
Trees within provenance	64.20 ($P=0.00$)	65.84 ($P=0.00$)	45.35 ($P=0.00$)	57.81 ($P=0.00$)	31.29 ($P=0.00$)	48.62 ($P=0.00$)
Within trees (error)	24.88	34.16	8.92	17.27	11.53	17.61
Total	100	100	100	100	100	100

Table 3
Matrix of correlations between DBH, measured and derived variables on fruit and components of *M. sylvatica*.

	Fruit Length	Fruit width	Fruit Weight	Peel Weight	Kernel Weight	Pulp Weight	DBH
Fruit Length	1,00						
Fruit width	0,91	1,00					
Fruit Weight	0,87	0,77	1,00				
Peel Weight	0,77	0,66	0,94	1,00			
Kernel Weight	0,81	0,72	0,97	0,90	1,00		
Pulp Weight	0,90	0,81	0,97	0,87	0,91	1,00	
DBH	-0,20	-0,01	-0,36	-0,42	-0,35	-0,32	1,00

Table 4Proximate composition of *M. sylvatica* fruit pulp and kernel.

Parameter	Nutritional profile of wild mango (<i>M. sylvatica</i>) pulp and kernel										
	Fibre (%)	Moisture (%)	Carbohydrate (%)	Ash (%)	Fat(%)	Protein (%)	Calorific value (KJ/100g)	Vitamin C (mg/100g)	Vitamin A (mg/100g)	Sugar (Brix ^o)	pH
Fruit pulp											
Sylhet	2.08 ±0.07 ^a	85.20 ±0.62 ^a	2.16±0.82 ^a	2.17 ±0.24 ^a	3.07 ±0.27 ^a	5.33 ±0.19 ^a	57.59 ±4.05 ^a	35.81 ±12.45 ^a	4.61 ±1.87 ^a	14.44 ±0.72 ^a	2.65 ±0.13 ^a
Cox's Bazar	2.34 ±0.15 ^a	85.46 ±1.13 ^a	1.74±1.00 ^a	1.58 ±0.28 ^a	3.40 ±0.25 ^a	5.05 ±0.29 ^a	59.47 ±2.90 ^a	52.88 ±10.75 ^a	1.34 ±1.00 ^a	13.04 ±1.27 ^a	2.66 ±0.09 ^a
Chittagong	2.14 ±0.06 ^a	85.29 ±1.21 ^a	2.00±0.83 ^a	2.09 ±0.30 ^a	3.43 ±0.22 ^a	5.48 ±0.60 ^a	59.09 ±4.32 ^a	52.86 ±5.50 ^a	2.63 ±1.01 ^a	11.30 ±1.94 ^a	2.65 ±0.13 ^a
Average	2.19 ±0.06	85.32 ±0.57	1.95±0.48	1.93 ±0.17	3.32 ±0.14	5.29 ±0.23	58.80 ±4.08	41.51 ±6.06	2.44 ±0.65	12.93 ±0.83	2.65 ±0.06
Fruit kernel											
Sylhet	9.99 ±0.95 ^a	44.91 ±4.63 ^a	28.41±5.54 ^a	0.06 ±0.03 ^a	11.42 ±0.45 ^a	5.21 ±0.19 ^a	237.20 ±21.22 ^a	NE	NE	NE	NE
Cox's Bazar	9.36 ±0.48 ^a	57.30 ±5.70 ^a	17.65±6.79 ^a	0.16 ±0.06 ^a	11.76 ±0.26 ^a	5.48 ±0.35 ^a	196.11 ±21.62 ^a	NE	NE	NE	NE
Chittagong	10.44 ±0.56 ^a	54.85 ±6.29 ^a	16.64±5.16 ^a	0.08 ±0.04 ^a	11.51 ±0.75 ^a	4.78 ±0.37 ^a	191.53 ±21.35 ^a	NE	NE	NE	NE
Average	9.93 ±0.37	52.89 ±3.37	20.36±3.47	0.10 ±0.03	11.57 ±0.29	5.15 ±0.20	206.22 ±17.26	NE	NE	NE	NE

important measurement in the processing, preservation and storage of food (Akpabio and Ikpe, 2013). *M. sylvatica* contains higher quantities of moisture, which is an indication of the low shelf life of the fruit, indicating that fresh fruit may not be able to be stored for long time due to its susceptibility to microbial attack (Ogunbanwo et al., 2013; Onwuka, 2014). However, *M. sylvatica* fruit has a wide range of variation in sugar (7 to 17 Brix^o) content (Supplementary Fig. 2). Fruits from the Sylhet site contained higher amounts of sugar where the climate is (temperature and rainfall) cooler compared to the Chittagong and Cox's Bazar sites (Zafer Siddik et al., 2013). Moreover, fruit phenotypic traits also support these findings, shown in Table 1 and Table 4. Similar findings are mentioned by Maranz and Wiesman (2003) for *Vitellaria paradoxa* in Africa. A high correlation was observed between sugar and Vitamin A (Table 4). It was realised that fruits from the Sylhet site hold higher amounts of Vitamin A compared to the other two sites (Table 4). Therefore, it can be concluded that fruits from the Sylhet site are sweeter and a rich source of Vitamin A, which can be considered during cultivar development for pulp production. On the other hand, a reasonable array of variation in kernel fat (9.00 to 14.00 %) was observed for *M. sylvatica* fruits, where higher fat content can be utilised in the food, pharmaceutical or cosmetic industries (Leakey et al., 2008). Our study demonstrates that fruits from the Cox's Bazar site, which is the hottest zone in terms of temperature and rain fall (Zafer Siddik et al., 2013) contained bigger kernels (Table 1) and higher fat percentages (Table 4) compared to the other two local sites. On the other hand, kernels with lower

saturated and unsaturated fat ratios (Fig. 3a), higher oleic and linoleic acid (Fig. 3b) and lower fat content can be suitable as edible kernels. Although there was no significant site variation in the fatty acid composition of *M. sylvatica* butter/fat, there are similarities with cocoa butter (Lipp and Anklam, 2008). Therefore, there is the potential for *M. sylvatica* butter to be used in the chocolate and confectionery industries as a partial or total replacement for cocoa butter. Thus, the domestication activity should not only contemplate the continuous variation but also take into account the resemblances in the population, and a higher level of selection pressure can be applied based on the ideotype selected (Leakey and Page, 2006). As the *M. sylvatica* tree produces fruits that have the potential to be used for pulp and kernel, this may be considered a new crop plant. For the utilisation of this rich wild fruit, it is necessary to develop novel foods from these fruits. Therefore, there is an urgent need for the food industry to work with foresters and horticulturists to domesticate this species, so that the process of genetic selection can include the traits important for the food industry. In contrast, the use of these fruits can lead to the initiation of the domestication processes by local farmers.

In the 21st century, the world has made much progress in reducing hunger, poverty and malnutrition. However, these problems, particularly in the developing world, are an unresolved issue. The number of undernourished people in developing countries was 780 million in 2014–16 (FAO, 2015). According to the WHO, one in three people in developing countries suffers from nutrition (vitamin and mineral)

Table 5Macro and micro mineral composition of *M. sylvatica* fruit pulp, kernel and peel.

Site	Mineral composition of <i>M. sylvatica</i> fruit					
	Na	K	Ca	Mn	Zn	Fe
Mango pulp (mg/100g)						
Cox's Bazar	34,15±11,90 ^a	175,21±22,07 ^a	16,83±2,78 ^{ab}	1,50±0,47 ^a	0,19±0,04 ^a	0,21±0,05 ^a
Chittagong	59,57±10,63 ^a	191,98±31,18 ^a	22,85±3,71 ^a	1,91±0,67 ^a	0,19±0,03 ^a	0,23±0,01 ^a
Sylhet	73,90±27,85 ^a	145,68±11,35 ^a	9,61±1,45 ^b	1,00±0,35 ^a	0,11±0,00 ^a	0,18±0,03 ^a
Average	54,58±9,97	172,76±13,98	16,92±2,15	1,50±0,30	0,17±0,02	0,21±0,02
Mango kernel (mg/100g)						
Cox's Bazar	9,62±2,10 ^a	84,57±7,50 ^a	8,20±1,69 ^a	0,77±0,17 ^a	0,17±0,02 ^a	0,89±0,16 ^a
Chittagong	13,47±4,92 ^a	75,14±6,02 ^{ab}	9,91±1,77 ^a	0,68±0,23 ^a	0,16±0,01 ^a	0,63±0,18 ^a
Sylhet	4,07±0,99 ^a	54,79±0,39 ^b	6,37±1,02 ^a	0,32±0,08 ^a	0,14±0,02 ^a	0,31±0,02 ^a
Average	9,41±2,07	72,69±4,61	8,29±0,94	0,61±0,11	0,16±0,01	0,63±0,10
Mango peel (mg/100g)						
Cox's Bazar	30,99±7,96 ^a	128,76±10,05 ^a	17,82±1,80 ^a	1,37±0,31 ^a	0,38±0,14 ^a	0,52±0,06 ^b
Chittagong	28,09±6,45 ^a	164,51±8,32 ^a	23,84±2,74 ^a	1,57±0,43 ^a	0,36±0,14 ^a	0,44±0,05 ^b
Sylhet (n=4)	43,57±13,03 ^a	176,81±21,62 ^a	28,27±8,31 ^a	1,65±0,56 ^a	0,20±0,02 ^a	0,95±0,07 ^a
Average	33,55±5,09	155,25±9,02	22,95±2,67	1,52±0,23	0,32±0,07	0,61±0,07

N.B. Macronutrients: Na K, Ca; Micronutrients: Zn, Mn, Fe; SE = Standard Error.

Table 6

Medicinal compounds detected (by HPLC-UV) in *M. sylvatica* and their medicinal use.

Medicinal compound in <i>M. sylvatica</i> pulp	Anti-cancer	Anti-diabetic	Anti-inflammatory	Prevention of liver steatosis	Anti-allergic	Anti-viral	Anti-microbial	Anti-ulcer	Heart diseases	Anti-coagulant	Anti-obese	Anti-mutagenic	Anti-osteoporotic	Anti-estrogenic	Neuroprotective	Anti-anxiety	Analgesic	Anti-angiogenic	Immunomodulatory	Reference
Mangiferin	✓	✓	✓	✓	✓	✓	✓			✓	✓						✓		✓	Benard and Chi, 2015; Mujawdiya and Kapur, 2015; Matkowski et al., 2013; Vyas et al., 2012; Masibo and He, 2008
Quercetin	✓		✓		✓	✓	✓		✓		✓	✓			✓					Hossen et al., 2015; Nabavi et al., 2015; Maalik et al., 2014; Asif and Khodadadi, 2013; Yoshida et al., 1990; Hollman et al., 1996
Kaempferol	✓	✓	✓		✓		✓	✓			✓		✓	✓	✓	✓	✓			Zang et al., 2015; Calderón-Montaña et al., 2011; Ackland et al., 2005
p-coumaric acid	✓	✓	✓					✓												Pei et al., 2016; Krishna et al., 2014; Srivastava et al., 2007
Gallic acid	✓		✓			✓	✓			✓	✓	✓						✓		Choubey et al., 2015; Pandey et al., 2014; Vazirian et al., 2011; Madsen and Bertelsen, 1995
Ellagic acid	✓		✓			✓					✓	✓								Kang, 2015; Park et al., 2014; Favarin et al., 2013; Usta et al., 2013; Mandal et al., 1988

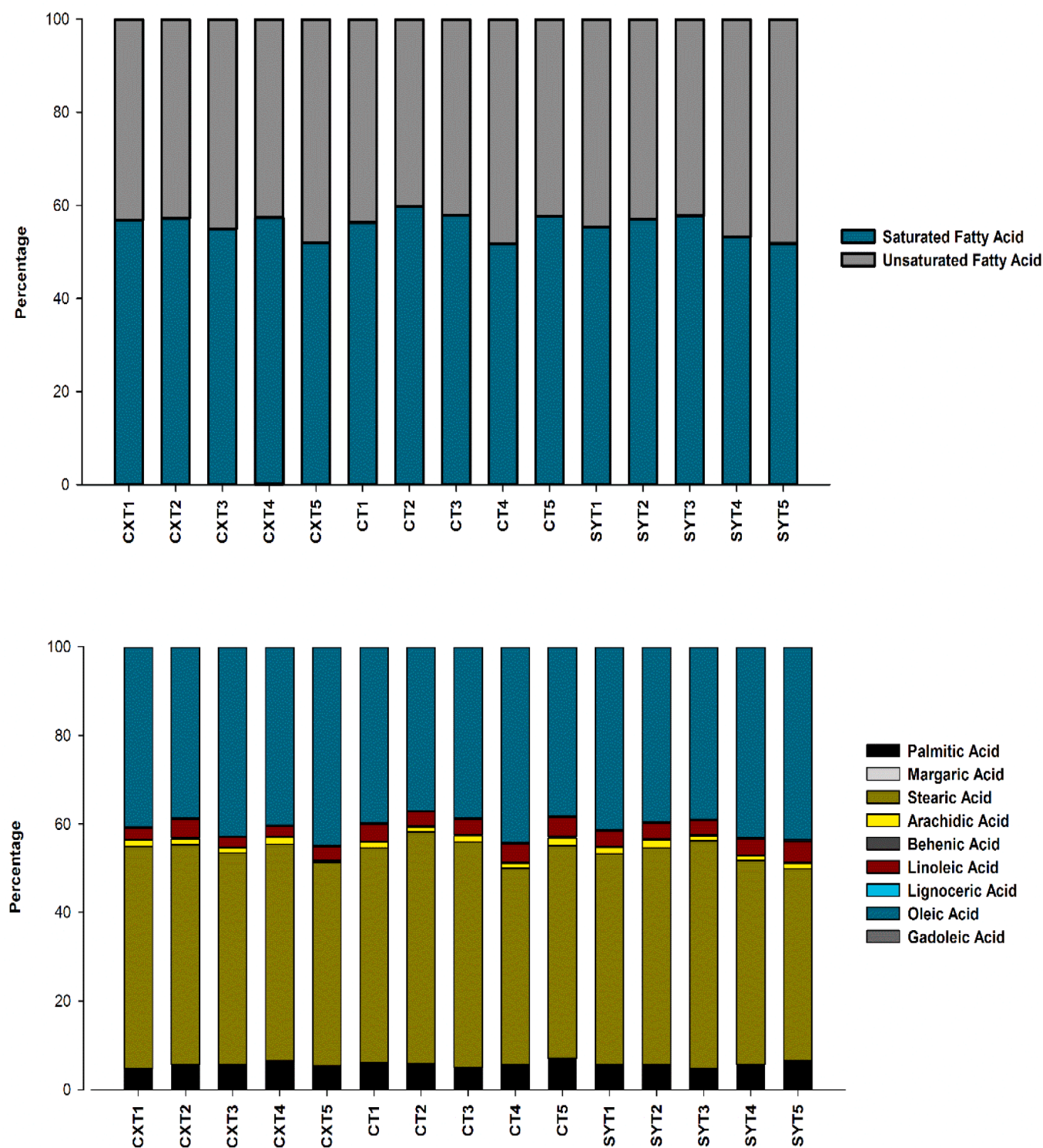


Fig. 3. (a) saturated and unsaturated lipid content; (b) fatty acid profile of *M. sylvatica* kernel.

deficiency. Every second pregnant woman and 40% of preschool children are estimated to be anaemic; and 250 million preschool children are Vitamin A deficient in developing countries (WHO, 2016). Wild fruits can play a crucial role in mitigating the effects of nutrient deficiencies and consumption of a variety of wild fruits can potentially contribute to health improvement (Judprasong et al., 2013). Wild fruit is an important source of food, medicine and income for forest dwellers, and tribal and marginalised rural people (Judprasong et al., 2013). In the rural

countryside of many developing nations, wild fruits are consumed as people cannot afford commercial, domesticated fruits (apple, grapes, pomegranate and orange). Some wild fruits have been identified as having better nutritional value than cultivated fruits (Mahapatra et al., 2012). They are nutritionally rich and traditionally used as supplements to the staple diet. Several wild foods are used only in times of scarcity and famine (FAO, 1992). Many of these famine foods have a higher protein, energy, vitamin (A and C) and mineral content than

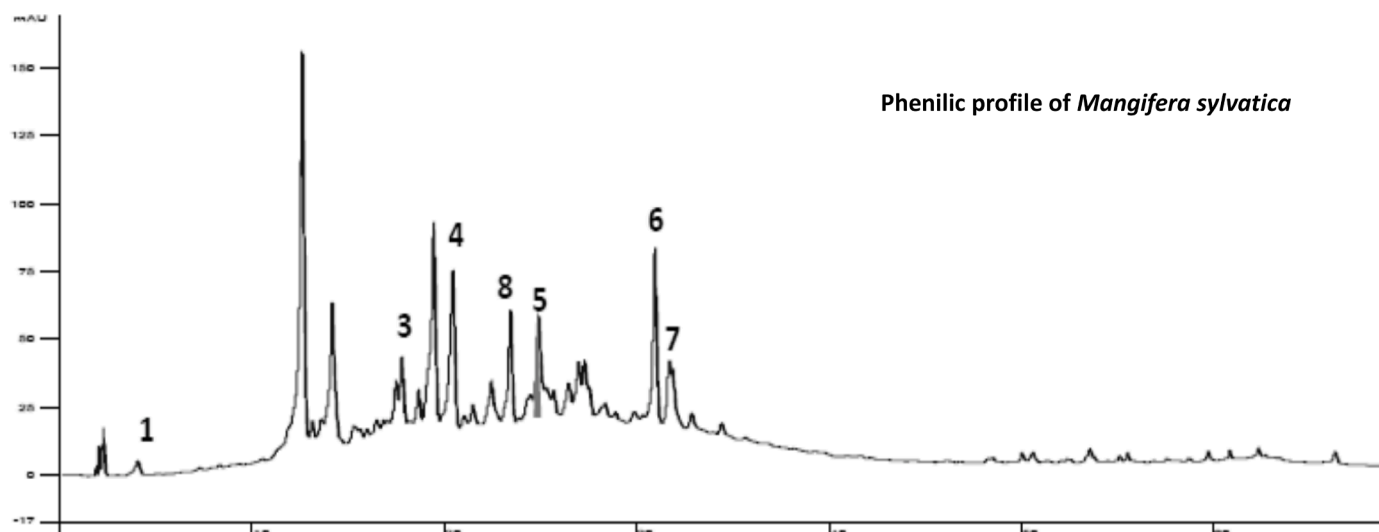
Phenolic profile of *Mangifera sylvatica*

Fig. 4. Phenolic finger printing of wild mango. Number indicates different type of phenolic compound. Gallic Acid (1), Syringic Acid (2), Mangiferin (3), P-Coumaric Acid (4), Benzoic Acid (5), Quercetin (6), Cinnamic Acid (7), Ellagic Acid (8).

domesticated varieties. Still, there are many wild fruits available in the forest that are underexploited and information on their nutritional values and medicinal properties, storage ability and economic potential are unknown, except for their consumption value and taste. As a result, in recent years, a growing interest has emerged to evaluate the edibility, nutritional features and therapeutic potential of various wild fruit species (Nazarudeen, 2010; Aberoum and Deokule, 2009) and other authors suggest an inventory of wild food resources may promote conservation of wild fruit species. *M. sylvatica* Roxb. is one such wild fruit species for which there has been no information to date on its morphological and nutritional value. The present study provides noteworthy information on the morphological traits and nutritional profile of the *M. sylvatica* species, which can open up opportunities for cultivar development that can assist in the commercialisation and conservation of this underutilised wild fruit species. For conservation of *M. sylvatica* morphological and nutritional traits should be utilised. It is clear from this study that fruit pulp and kernels even from a small population of individual trees of *M. sylvatica* vary considerably in morphological and nutritional traits, although the site variation was not great. This variability provides excellent opportunities for the domestication of the species through the development of multiple-trait cultivars from elite trees, using simple horticultural techniques (Leakey et al., 2008). So, if we can conserve the *M. sylvatica* species, this can help to combat nutritional deficiency at local levels and village-level domestication can potentially enhance the livelihoods of local people, while maintaining genetic diversity at the national level (Leakey and Page, 2006; Leakey et al., 2003). The current study can promote the development of a strategy for domestication of *M. sylvatica* to empower smallholders and enhance their livelihoods and income. This strategy aims to fulfil the demand of producers and consumers and offers opportunities for wider-scale marketing of *M. sylvatica* fruits and kernels.

5. Conclusion

M. sylvatica is a wild species that could be used as an alternative source of nutrition to satisfy hidden hunger. Some interesting and valuable morphological and nutritional information has come to light that can be used as a tool for nutrition education and to encourage people to consume *M. sylvatica* fruit. The current study may lead to the beginning of a domestication programme of this wild underutilised fruit species. The results also highlight a continuous variation in fruit traits, which are important for consumption and market-orientated selection. The kernel of this wild fruit species is a good source of mango butter that

can be utilised, although further research on this sector is also needed. Additionally, contribution from the food industry is also required to identify potentially novel food products. Moreover, research on the agroforestry suitability of this species would also be necessary for adequate and appropriate conservation of this wild species. This paper calls for greater collaboration between foresters, horticulturists and the food industry to promote the conservation and commercialisation of the *M. sylvatica* fruits in Bangladesh.

Conflict of Interest

Authors declare that there is no conflict of interest.

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Supplementary materials

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